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(54) Title: SPRAY-DRIED AMYLASE, PHARMACEUTICAL PREPARATIONS COMPRISING THE SAME AND USE

(57) Abstract: The present application relates to methods for the manufacture of pharmaceutical compositions comprising stabilized enzymes, especially amylase, lipase and protease, obtained by spray-drying in the presence of enzyme stabilizing agents resulting in particles comprising homogenously distributed molecular mixtures of said enzyme and said enzyme stabilizing agent. Furthermore the application describes pharmaceutical compositions containing said stabilized enzymes in the form of pellets which are particles comprising a core particle, which is coated with said stabilized enzyme. These pellets additionally can be coated with further substances. Furthermore the invention comprises pharmaceutical compositions prepared with said methods as well as the use of said pharmaceutical compositions for preparation of medicaments as well as their use for treatment of diseases or disorders such as digestive disorders, pancreatic exocrine insufficiency, pancreatitis, cystic fibrosis, diabetes type I and diabetes type II.

Spray-dried Amylase, Pharmaceutical Preparations Comprising The Same And Use

TECHNICAL FIELD OF THE INVENTION

The present application relates to methods for the manufacture of pharmaceutical compositions comprising amylase which has been spray-dried in the presence of an amylase-stabilizing agent, thereby increasing the yield of active amylase in the intermediate spray-dried amylase and in the final product made with said spray-dried amylase. These pharmaceutical compositions may contain said amylase in the form of pellets which optionally may be non-pareil pellets coated with said amylase. These pellets can be coated with additional substances. Furthermore the invention comprises pharmaceutical compositions prepared using said methods as well as the use of said pharmaceutical compositions for preparation of medicaments as well as their use for treatment of diseases or disorders such as digestive disorders, pancreatic exocrine insufficiency, pancreatitis, cystic fibrosis, diabetes type I and diabetes type II.

BACKGROUND OF THE INVENTION

Several commercial medicaments in the form of pancreatic enzyme supplements are known for the treatment of pancreatic exocrine insufficiency. The active ingredients of these products are digestive enzymes, mainly amylase, lipase and protease, which are normally produced in the pancreas and which are needed for digestion of sugar (amylase), fat (lipase), and protein (protease). These enzymes are normally excreted to the upper part of the small intestine, the duodenum. The enzymes used in such medicaments commonly are derived from bovine or swine pancreas, however there are also products on the market containing microbial enzymes, e.g. the product Nortase® which contains a lipase from Rhizopus oryzae, a protease from Aspergillus oryzae, and an amylase from Aspergillus oryzae.

Pancreatic enzyme supplements show optimal stability under near neutral and slightly alkaline conditions. Consequently these enzymes might suffer partial loss of ac-

tivity during gastric-passage. Therefore exogenously administered enzymes often are protected against gastric inactivation by enteric coatings. However uncoated exogenously administered enzyme preparations are also sold as medicaments. After passage through the stomach the enzymes should be released into the upper duodenum in order to function as intended, e.g. in order to digest metabolites such as sugar, fat and protein.

WO 06/136161 describes the pharmaceutical use of certain amylases for the treatment of digestive disorders and other diseases.

As the registration process for medicaments is very strict and safety data must be provided for all active ingredients thereof as well as for any by-products like degradation products. It is therefore preferred to produce medicaments with a high purity, with a high content of active ingredient and with the lowest possible content of by-products, e.g. by-products from degradation. Therefore amylase-containing medicaments ideally should contain amylase of the highest possible specific activity and with the lowest possible amount of aggregated or inactive forms of amylase.

Commercially available digestive enzyme compositions show a loss of enzymatic activity during storage and therefore manufacturers typically overfill the dosage forms to compensate for this loss in activity during storage. WO 2008/102264 describes compositions comprising digestive enzymes, wherein the composition has a moisture content of about 3% or less. According to WO 2008/102264 this serves to provide a more stable composition, as compared to compositions having a moisture content above 3%.

However loss of activity of enzymes not only occurs during storage of the final product such as an enzyme-containing medicament, but loss of enzyme activity may already occur during the manufacturing process. This is a separate issue besides the stability-issue of the final product.

SUMMARY OF THE INVENTION

The present invention provides a pharmaceutical composition comprising a spray-dried mixture of amylase and at least one amylase-stabilizing agent, said spray-dried mixture being obtainable by spray-drying a liquid comprising an amylase and at least one amylase-stabilizing agent, wherein said amylase shows at least 85% of the specific amylase activity relative to the specific amylase activity before spray-drying.

Furthermore the invention comprises said pharmaceutical composition in the form of a powder , in the form of a granulate, in the form of pellets or in the form of a coating on a core particle, which pharmaceutical composition furthermore is processed into a tablet, a capsule or a sachet.

The amylase-stabilizing agent according to the invention preferably is a non-reducing carbohydrate, preferably a polysaccharide, an oligosaccharide, a disaccharide, a sugar-alcohol, or a cyclodextrin, wherein said polysaccharide is selected from Inulin, or Dextran, preferably Dextran 1, Dextran 40 or Dextran 70, said oligosaccharide selected from Sucrose or Trehalose, said sugar-alcohol selected from Sorbitol, Mannitol, or Maltitol, said cyclodextrin is Hydroxyl-Propyl beta Cyclodextrin, and said enzyme stabilizing agent is selected can also be Gycine, Arginine or a mixture of Calcium salts and Methionine.

The amylase use according to the invention preferably is selected from alpha-amylase, beta-amylase, alpha-glucosidase, isoamylase, glucan 1,4-alpha-maltohydrolase, preferably an amylase originating from bacteria, yeast, mammalian or human cells, preferably an amylase produced by recombinant technologies.

The pharmaceutical composition according to the invention preferably contains amylase which is purified and has a purity of at least 90 area-% (w/w) and may furthermore comprise a lipase and/or a protease.

Pellets, granulates, coated core-particles or tablets prepared from the spray-dried amylase according to the invention can be coated with at least one layer of a pharmaceutically acceptable substance, wherein said at least one layer preferably is a functional coating or a non-functional coating. The functional coating preferably represents an en-

teric coating protecting the enzymes of the pellets from acid pH during gastric passage, and the non-functional coating is preferably a coating preventing the formation of dust from the material positioned below said non-functional coating.

Furthermore the invention comprises methods for the manufacture of a pharmaceutical composition according to claim 1 comprising the step of spray-drying an amylase solution mixed with at least one amylase-stabilizing agent prior to spray-drying, which method results in a pharmaceutical composition comprising an amylase showing at least 85% specific amylase activity relative to the specific amylase activity prior to spray-drying.

Furthermore the invention comprises methods for the manufacture of a pharmaceutical composition comprising the step of coating pharmaceutically acceptable core particles with a coating solution comprising amylase, wherein said coating solution is prepared by mixing of an amylase solution with at least one enzyme stabilizing agent and optionally coating said core particles with one or more further coating layer comprising amylase and/or other pharmaceutically acceptable substances and incorporating said coated core pellets into a pharmaceutical composition.
In this method the amylase is not spray-dried prior to preparation of the coating solution, but the coating solution is prepared directly from the liquid amylase preparation as purified, and the amylase-stabilizing agent is added to the amylase liquid concentrate prior to coating the core particles with said coating solution.

Preferably in this method said coating solution is prepared from a spray-dried amylase according to the invention (e.g. amylase mixed with at least one amylase-stabilizing agent and subsequently spray-dried) and a solvent.

Furthermore the invention comprises a method for the manufacture of a pharmaceutical composition comprising the step of preparing granulates, pellets, coated core particles, tablets, capsules or sachets from the spray-dried amylase of the invention.

Furthermore the invention comprises pharmaceutical composition containing spray-dried amylase of the invention for the prevention or treatment of digestive disor-

ders, pancreatic exocrine insufficiency, pancreatitis, cystic fibrosis, maldigestion, diabetes type I or diabetes type II.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Time-dependant formation of aggregates at t=0 (A and C) and at t=4 weeks (B and D) of spray-dried amylase without excipient (A and B) and with trehalose (1:1, w/w) (C and D)

Figure 2: Exemplary chromatogram for determination of amylase purity and content according to Example 4. Figure 2 A shows the complete chromatogram, whereas Figure 2 B shows only part of the chromatogram in an enlarged scale, in order to make easier visible some peaks originating from impurities present in the analyzed sample.

Figure 3: Exemplary reaction scheme of the Maillard reaction according to Example 10. The carbonyl group of the sugar reacts with the amino group of the amino acid, resulting in N-substituted glycosylamine and water.

DETAILED DESCRIPTION OF THE INVENTION

Amylase

In what follows, the amylase for use in the compositions, methods and uses of the invention is referred to as the "amylase of the invention". In the present context, an amylase is an enzyme that catalyzes the endo-hydrolysis of starch and other linear and branched oligo- and polysaccharides. In a particular embodiment, the amylase for use according to the invention has alpha-amylase activity, viz. catalyzes the endohydrolysis of 1,4-alpha-glucosidic linkages in oligosaccharides and polysaccharides. Alpha-amylases act, e.g., on starch, glycogen and related polysaccharides and oligosaccharides in a random manner, liberating reducing groups in the alpha-configuration.

In a preferred embodiment the amylase of the invention is an alpha-amylase (systematical name: 1,4-alpha-D-glucan glucanohydrolase). In further embodiments, the amylase of the invention belongs to the EC 3.2.1.- group of amylases, such as EC 3.2.1.1 (alpha-amylase), EC 3.2.1.2 (beta-amylase), EC 3.2.1.3 (glucan 1,4-alpha-

glucosidase, amyloglucosidase, or glucoamylase), EC 3.2.1.20 (alpha-glucosidase), EC 3.2.1.60 (glucan 1,4-alpha-maltotetraohydrolase), EC 3.2.1.68 (isoamylase), EC 3.2.1.98 (glucan 1,4-alpha-maltohexosidase), or EC 3.2.1.133 (glucan 1,4-alpha-maltohydrolase). In a preferred embodiment, the amylase for use according to the invention can be, or is, classified as belonging to the EC 3.2.1.1 group. The EC numbers refer to Enzyme Nomenclature 1992 from NC-IUBMB, Academic Press, San Diego, California, including supplements 1-5 published in Eur. J. Biochem. 1994, 223, 1-5; Eur. J. Biochem. 1995, 232, 1-6; Eur. J. Biochem. 1996, 237, 1-5; Eur. J. Biochem. 1997, 250, 1-6; and Eur. J. Biochem. 1999, 264, 610-650; respectively. The nomenclature is regularly supplemented and updated; see e.g. the World Wide Web at http://www.chem.qmw.ac.uk/iubmb/enzyme/index.html.

In a further particular embodiment, the amylase of the invention comprises at least one substitution, deletion, and/or insertion of one or more amino acids in any one of the sequences. Preferably, the amino acid changes are of a minor nature, that is conservative amino acid substitutions or insertions that do not significantly affect the folding and/or activity of the protein; small deletions; small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue; a small linker peptide; or a small extension that facilitates purification by changing net charge or another function, such as a poly-histidine-tag, an antigenic epitope or a binding domain.

In still further particular embodiments of the invention, the amylase is derived from a microorganism, for example from a fungus, or from a bacterium. Examples of bacteria are strains of Bacillus, such as strains of Bacillus amyloliquefaciens, Bacillus circulans, Bacillus halmapalus, Bacillus licheniformis, Bacillus megaterium, Bacillus sp., Bacillus stearothermophilus, and Bacillus subtilis; preferably from strains of Bacillus amyloliquefaciens, Bacillus circulans, Bacillus halmapalus, Bacillus licheniformis, Bacillus megaterium, Bacillus sp., and Bacillus stearothermophilus; most preferably from strains of Bacillus stearothermophilus, Bacillus licheniformis, or Bacillus sp. In this context, the term “derived from” includes enzymes obtainable, or obtained, from wildtype strains; as well as, preferably, variants thereof having at least one substitution, insertion, and/or deletion of at least one amino acid residue. The term variant also includes shufflants, hybrids, chimeric enzymes and consensus enzymes. The variants may have been produced by any method known in the art, such as site-directed mutagenesis, random

mutagenesis, consensus derivation processes (EP 897985), and gene shuffling (WO 95/22625, WO 96/00343), etc.

Further particular examples of amylases of the invention are the amylases contained in commercial products such as Clarase, DexLo, GC 262 SP, G-Zyme G990, G-Zyme G995, G-Zyme G997, G-Zyme G998, HTAA, Optimax 7525, Purastar OxAm, Purastar ST, Spezyme AA, Spezyme Alpha, Spezyme BBA, Spezyme Delta AA, Spezyme DBA, Spezyme Ethyl, Spezyme Fred (GC521), Spezyme HPA, and Ultraphlow (all from Genencor); Validase BAA, Validase FAA, Validase HT340L, Valley Thin 340L (all from Valley Research); Avizyme 1500, Dextro 300 L, Kleistase, Maltazyme, Maxamyl, Thermozyme, Thermatex, Starzyme HT 120 L, Starzyme Super Conc, and Ultraphlo.

In still further particular embodiments, an additional amylase may be used. Examples of additional amylases are mammalian amylases, and microbial proteases. A preferred mammalian amylase is pancreas extract, e.g. from swine or ox, such as pancreatin. Preferably said mammalian amylase from pancreas extract is purified to contain at least 20%, preferably at least 30%, preferably at least 40%, preferably at least 50%, preferably at least 60%, preferably at least 70%, preferably at least 80%, preferably at least 90%, preferably at least 95%, preferably at least 98% of Amylase protein as calculated based on the total amount of protein present in said pancreas extract. The Amylase protein content and purity can be determined for example using the methods described in Example 4.

Preferably the amylase of the invention is purified by methods known in the art. Isolation, purification, and concentration of the enzyme(s) of the invention may be carried out by conventional means. For example, the amylase may be recovered from a fermentation broth by conventional procedures including, but not limited to, centrifugation, filtration, ultrafiltration, dialfiltratino, extraction, spray-drying, evaporation, or precipitation, and further purified by a variety of procedures known in the art including, but not limited to, chromatography (e.g., ion exchange chromatography, affinity chromatography, hydrophobic interaction chromatography, reverse phase chromatography, chromatofocusing, isoelectric focusing and size exclusion chromatography, etc.), electrophoretic procedures (e.g., preparative isoelectric focusing), differential solubility (e.g., ammonium sulphate precipitation), SDS-PAGE, or extraction (see, e.g., Protein Purification, J.-C. Janson and

Lars Ryden, editors, VCH Publishers, New York, 1989). The resulting purified amylase preferably has a purity, which for example can be determined as described in Example 4 which is at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the total protein present in the purified amylase sample. These percentage values refer only to the protein content and do not include salts, sugars, lipids etc. which might be present as impurities in the purified amylase preparation. Preferably these percentage values also include non-protein impurities such as salts, sugars, lipids, etc.

Preferably the amylase of the invention has after spray-drying a amylase protein content of at least 10%, 20%, 30%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90%. In general after spray-drying the amylase of the invention has a lower amylase protein content as compared to the same amylase prior to spray-drying, because prior to spray-drying amylase-stabilizing agents according to the invention are added to the amylase, resulting in a lower relative amylase protein content of the resulting spray-dried amylase.

In particular embodiments, the amylase of the invention is an amylase as described in WO9310249, WO9623873, WO9741213 or WO 2006/136161.

Preferably the amylase is a thermostable amylase. An amylase is regarded as a thermostable amylase according to the invention, if at least 50 %, preferably 55%, preferably 60%, preferably 65, preferably 70%, preferably 75%, preferably 80%, preferably 85%, preferably 90%, preferably 95%, preferably 98% of its activity is preserved if the enzyme is dissolved in amylase-activity assay buffer according to Example 1 and is subjected to an increased temperature equal or above 50°C for 1 hour. The increased temperature for determining thermostability of an amylase preferably is 50°C, preferably 55°C, preferably 60C°, preferably 65°C, preferably 70°C, preferably 75°C, preferably 80C°, preferably 85°C, prferably 90°C, preferably 95°C, preferably 100°C. Alternatively an amylase is regarded as a thermostable amylase, if said amylase is naturally present in a thermostable microorganism, such as a thermostable bacteria, wherein said thermostable microorganism are defined as microorganism able to proliferate at about normal rates at temperatures above 50°C. A proliferation rate is regarded as “about normal” if

the doubling time of the number of individuals of said microorganism is hat least 20% of its fastest doubling time under optimal growth conditions.

Specific amylase activity

The specific amylase activity can be measured using amylase assays as known in the art. The specific activity in units/gram [U/g] is determined by dividing the total amylase activity through the total amount of amylase protein. The amylase activity preferably is determined using the method of example 1. Alternatively the amylase activity might be determined according to the protocol of the European Pharmacopoeia 6.3, page 4260 – 4263, or using colorimetric assays known in the art, which are measuring amylase activity.

Preferably the amylase of the invention shows at least 85%, preferably 90, preferably 95% specific activity. The variation of the measured values due to errors preferably is +/- 10%, more preferably 10%. The variation of the measured value for the specific amylase activity before spray-drying preferably is +/- 10%. Preferably the amylase of the invention shows at least 85% to 100%, preferably 90% to 100%, most preferably 95% to 100% of the specific amylase activity before spray-drying. Preferably the amylase of the invention shows at least 85% +/-5% to 100% +/-10%, preferably 90% +/-5% to 100% +/-10%, most preferably 95% +/-5% to 100% +/-10% of the specific amylase activity before spray-drying. Preferably the amylase of the invention shows at least 90% +/-10% to 100% +/-10%, most preferably 95% +/-10% to 100% +/-10% of the specific amylase activity before spray-drying.

The total amount of amylase protein is preferably determined by the method of Example 4. Alternatively also the method according to Example 3 might be used for determination of the amount of amylase protein. There are further methods known in the art to determine the amount of amylase protein such as amylase-specific Enzyme-Linked-Immunosorbent Assays (ELISA), quantitative western blotting by e.g use of chemiluminesce detection methods, use of bio-chips specific for amylase, etc. The content of amylase present in an peak of a chromatogram may be determined as described in Example 3 or in Example 4 but alternatively the eluate of said amylase peak might also be ana-

lyzed using a standard protein assay as known in the art such as the Bradford protein assay (Bradford, M., Analytical Biochemistry, 1976, volume 72, pages 248-254).

Protease

The amylase of the invention may be used, with or without a protease as mentioned elsewhere in this application. The term "protease" is defined herein as an enzyme that hydrolyses peptide bonds. It includes any enzyme belonging to the EC 3.4 enzyme group (including each of the thirteen subclasses thereof, these enzymes being in the following referred to as "belonging to the EC 3.4.-.- group").

The protease may be a mammalian proteases, or a microbial proteases. A preferred mammalian protease is pancreas extract, e.g. from swine or ox, such as pancreatin. The microbial protease may be, e.g., based on or derived from bacterial or fungal strains. The protease may in particular be derived from a strain of Aspergillus, such as Aspergillus oryzae or Aspergillus melleus, in particular the product Prozyme 6™ (neutral, alkaline protease EC 3.4.21.63) which is commercially available from Amano Pharmaceuticals, Japan. Examples of bacterial proteases are proteases from Bacillus and Nocardiopsis, such as the Bacillus licheniformis protease.

Preferably the protease may be a protease as described in WO05115445, or WO06136160.

Lipase

The amylase of the invention may be used in combination with a lipase. In the present context, a lipase means a carboxylic ester hydrolase EC 3.1.1.-, which includes activities such as EC 3.1.1.3 triacylglycerol lipase, EC 3.1.1.4 phospholipase A1, EC 3.1.1.5 lysophospholipase, EC 3.1.1.26 galactolipase, EC 3.1.1.32 phospholipase A1, EC 3.1.1.73 feruloyl esterase. In a particular embodiment, the lipase is an EC 3.1.1.3 triacylglycerol lipase.

The lipase may be a mammalian lipase, or a microbial lipase. A preferred mammalian lipase is pancreas extract, e.g. from swine or ox, such as pancreatin. The microbial lipase may, for example, be derived from bacterial or fungal strains, such as Bacillus, Pseudomonas, Aspergillus, or Rhizopus. The lipase may in particular be derived from a

strain of Rhizopus, such as Rhizopus javanicus, Rhizopus oryzae, or Rhizopus delemar, for example the product Lipase D Amano 2000™ (also designated Lipase D2™) which is commercially available from Amano Pharmaceuticals, Japan.

In further particular embodiments, the lipase is a recombinantly produced microbial lipase, for example derived from a fungus such as Humicola or Rhizomucor, from a yeast such as Candida, or from a bacterium such as Pseudomonas. In a preferred embodiment, the lipase is derived from a strain of Humicola lanuginosa or Rhizomucor miehei.

Preferably the lipase may be a lipase as described in EP0305216B1, WO9118623, WO9205249, WO9522615, WO9707202, WO9422903, WO0032758, WO0060063 or WO06136159.

Enzyme stabilizing agent

Suitable "enzyme stabilizing agents" for use with the coating layer(s) or with other elements of the pharmaceutical compositions described herein e.g. are non-reducing agents, in particular non-reducing carbohydrates, preferably non-reducing polysaccharides, non-reducing oligosaccharides, non-reducing disaccharides, non-reducing sugar-alcohols or a non-reducing cyclodextrine. Furthermore non-reducing substances such as Glycine, Arginine or mixtures of Calcium salts and Methionin are preferred enzyme stabilizing agents.

Non-reducing carbohydrates are carbohydrates that lack the ability to form aldehyde or ketone groups in basic solution. For this reason non-reducing carbohydrates cannot act as reducing agent, for example in the Maillard reacton.

Carbohydrates are only regarded as non-reducing if they completely lack any sugar building blocks representing reducing sugar building blocks. There are known carbohydrates, comprising non-reducing as well as reducing sugar building blocks. Such sugars are, according to the invention, regarded as reducing sugars, because part of their structure represents reducing sugars. An example of such a sugar is Maltodextrin, which for this reason is not regarded as a non-reducing carbohydrate but as a reducing carbohydrate. Maltodextin is able to react in a Maillard reaction.

Polysaccharides are carbohydrates composed of more than ten monosaccharide residues joined together by glycosidic bonds, Oligosaccharides are regarded as carbohydrates composed of two to ten monosaccharide residues, disaccharides are consequently a subgroup of oligosaccharides composed of two monosaccharide residues.

Preferred non-reducing polysaccharides are Dextran, preferably Dextran 1, Dextran 40 and Dextran 70, and non-reducing oligosaccharides are preferably Sucrose and Trehalose, and non-reducing sugar-alcohols are preferably Sorbitol, Mannitol, and Maltitol, and non-reducing cyclodextrines is preferably Hydroxyl-Propyl beta Cyclodextrin.

Preferably at least one enzyme stabilizing agent is added to the amylase sample prior to drying said amylase. The dried amylase is subsequently used to prepare a coating solution which is used for coating the core particles resulting in enzyme-coated pellets. During the coating process of the core particles the amylase present in the coating solution is dried fort the second time.

Alternatively the amylase sample is present in solution, the enzyme stabilizing agent is added to the amylase sulution and optional other substances and solvents are added, resulting in a coating solution without prior drying of the amylase. This coating solution is directly used for coating the core particles during which process the amylase present in the coating solution is dried while sticking already to the surface of the core particles.

Usually the enzyme stabilizing agents are mixed with the amylase in a mass:mass ratio of amylase:amylase-stabilizing agent of 1:0.025 to 1:5 preferably in a mass ratio of 1:0.1 to 1:3 more preferably in a mass ratio of 1:0.5 to 1:2. A mass ratio of for example 1:2 means that 1 g amylase and 2 g amylase-stabilizing agent are present in the composition. The amylase mass is always represented by the first number of rations written in a form like "1:2". Preferably the lower ration limit is 1:0.005, 1:0.01, 1:0.015, 1:0.02, 1:0.025, 1:0.03, 1:0.04, 1:0.05, 1:0.06, 1:0.07, 1:0.08, 1:0.09, 1:0.1, 1:0.15, 1:0.2,

1:0.25, 1:0.30, 1:0.35, 1:0.40, 1:0.45, 1:0.5, 1:0.6, 1:0.7, 1:0.8, 1:0.9, 1:1, 1:1.2, 1:1.4, 1:1.6, 1:1.8, 1:2, 1:2.5 or 1:3. Preferably the upper ratio limit is 1:0.05, 1:0.06, 1:0.07, 1:0.08, 1:0.09, 1:0.1, 1:0.15, 1:0.2, 1:0.25, 1:0.30, 1:0.35, 1:0.40, 1:0.45, 1:0.5, 1:0.6, 1:0.7, 1:0.8, 1:0.9, 1:1, 1:1.2, 1:1.4, 1:1.6, 1:1.8, 1:2, 1:2.5, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9 or 1:10. The optimal mass ratio between amylase and amylase-stabilizing agent depends on factors such as the type of amylase-stabilizing and the optionally further pharmaceutically excipients which might be present in the amylase formulation. Different enzyme stabilizing agents preferably are used in a different molar ratio to the amylase, based on the specific properties of these enzyme stabilizing agents. A too low ration of amylase:enzyme stabilizing agent may lead to an insufficient amylase-stabilizing activity of the enzyme stabilizing agent. Furthermore too much enzyme stabilizing agent is simply unnecessarily increasing the cost of the pellets and unnecessarily reducing the amount of amylase present in a certain amount of the final pellets (e.g. the relative amount of amylase within the pellets). Table 1 below indicates the preferred ratios of amylase and certain enzyme stabilizing agents:

Table 1: preferred mass ratios of amylase:amylase-stabilizing agent

Enzyme stabilizing agent	Amylase:enzyme stabilizing agent ratio
Succrose	1:0.5 or 1:1 or 1:2 or 1:3
Sorbitol	1:1
Maltitol	1:0.5 or 1:1 or, 1:2 or 1:3
Dextran 1	1:0.5 or 1:1 or 1:2 or 1:3 or higher ratios
Hydroxyl-Propyl beta Cyclodextrin	1:0.1 or 1:0.2 or 1:0.5 or 1:1 or higher ratios
Methionin	1:0.125 or 1:0.25 or 1:0.5 or 1:1
Glycin	1:0.5 or 1:1 or 1:2 or 1:3 or higher ratios
Arginin	1:0.5 or 1:1

Pellets

A pellet as described herein is usually obtained as a particle of spherical or nearly spherical shape, the shape being mainly due to the related manufacturing process. The size of pellets may vary in a broad range, but usually a diameter of at least 100, preferably of at least 200 micro meters is used, in particular where the pellets are for pharmaceutical use. More preferred for pharmaceutical use are pellets of a diameter of 200 to 4.000 micro meter, yet more preferred of 300 to 3.000 micro meter, still more preferred of 400 to 2.000 micro meter. Preferred lower limits of the diameter of a pellt are 100, 200, 300, 400, 500, 700, 1000, 2000, or 3000 micrometer. Preferred upper limits of the diameter of pellets are 200, 300, 400, 500, 700, 1000, 2000, 3000, or 4000 micrometer. A special form of pellets are coated non-pareil pellets, which comprise a core pellet (= neutral pellet = non-pareil pellet), which core pellet is coated with a pharmaceutical substance or composition and may comprise one or more than one coating layer.

Spray-drying

Spray-drying can be done as known in the art. Suitable inlet temperatures for spray-drying of amylase are in the range of 100 to 200°C. Suitable outlet temperature are in the range of 60 to 90°C. The outlet temperature is dependant on the inlet-temperature and the desired feeding rate (feed ratio speed). The difference of the inlet and the outlet temperature is proportional to the feeding rate. So for example the outlet temperature can be adjusted by changing the feeding rate. Preferably if the inlet temperature is about 100°C the corresponding outlet temperature is about 60 to 90°C, preferably if the inlet temperature is 120°C the outlet temperature is 60 to 90°C, preferably if the inlet temperature is 140°C the outlet temperature is 65 to 90°C, preferably if the inlet temperature is 160°C the outlet temperature is 70 to 90°C, preferably if the inlet temperature is 180°C the outlet temperature is 75 to 90°C, preferably if the inlet temperature is 200°C the outlet temperature is 85 to 90°C. Preferably the inlet temperature is regulated with an accuracy of plus/minus 10°C, preferably the outlet temperature is regulated with an accuracy of plus/minus 3°C.

Characteristics of spray-dried amylase

Spray-dried amylase forms highly porous particles with a consequently very high BET-surface area, as compared to other amylase drying techniques (BET = Brunauer, Emmet and Teller method). Particles of spray-dried proteins have a characteristic appearance, which distinguishes them form powder-material of proteins dried using other

methods. Particles of spray-dried proteins typically are irregular shaped, have a non uniform size distribution and display blowholes and cracks. These characteristics of spray-dried protein particles can be seen in Fig. 6 and Fig. 7 of Samborska et al., Drying Technology, 2005, Vol.: 23, page 950. There are various methods known in the art to characterize the physical properties of particles of spray-dried proteins, such as determining their BET-Surface Area for example using the gas sorption method (for example using a surface area analyzer of the Gemini™ VII 2390 Series or the ASAP™ 2020 or the TriStar™ II 3020 Surface Area and Porosimetry Systems, Micromeritics Instroment Corporation, Norcross, GA, USA). BET-Surface Area measurements determine the porosity of a material by measuring the amount of a gas needed to cover the surface and to fill the pores of the material to be tested. Other methods to determine porosity are mercury intrusion porosimetry which measures the pressure needed to intrude mercury into a sample's pores, which pressure is inversely proportional to the size of the pores, for examples using the AutoPore™ IV Series Mercury Porosimeters of Micromeritics. Furthermore particle size can be determined by commercially available instruments which for example are employing light scattering (for example using a laser particle size analyzer such as a Saturn DigiSizer® 5200, Micromeritics Instrument Corp.), employing the electrical sensing zone method (for example using an Elzone™ II 5390 particle sizing and counting analyzer, Micromeritics Instrument Corp.) or which are employing as measurement principle the sedimentation of the particles (for example using a SediGraph™ III 5120 particle size analyzer, Micromeritics Instrument Corp.) which measures mass by X-ray absorption and particle size by sedimentation. A brochure describing these methods and analyzing machines is available from Micromeritics Instrument Corp (brochure "Family of Products Brochure" downloaded on 01 July 2010 from http://www.micromeritics.com, direct link to PDF of brochure: http://www.micromeritics.com/Repository/Files/Family_Brochure_2009.pdf, product code from Mircomeritics for this brochure is 0309-SFC.

The spray-dried amylase particles preferably have a surface area of 1 to 50 m2/g (m2/g means square meter surface area per gram of particles), preferably 1 to 40 m2/g, preferably 1 to 30 m2/g, preferably 1 to 20 m2/g. The lower limit of the surface area of the particles is preferably 1 m2/g, preferably 2 m2/g, preferably 3 m2/g, preferably 4 m2/g, preferably 5 m2/g, preferably 6 m2/g, preferably 7 m2/g, preferably 8 m2/g, preferably 9 m2/g, preferably 10 m2/g. The upper limit of the surface area of the particles is

preferably 50 m2/g, preferably 45 m2/g, preferably 40 m2/g, preferably 35 m2/g, preferably 30 m2/g, preferably 25 m2/g, preferably 20 m2/g, preferably 15 m2/g, preferably 10 m2/g, preferably 5 m2/g.

Core particles

The "core particles" of the pharmaceutical compositions as described herein are by themselves usually pharmaceutically inactive and only function as carriers for the active pharmaceutical ingredient of a pharmaceutical composition, e.g. the amylase. Any type of pharmaceutically acceptable core particles known in the art for such purpose may be used, e.g. so called "non-pareil seeds" which are also sometimes referred to as "neutral pellets" or "starter pellets". The core particles may consist of any pharmaceutically acceptable organic or inorganic material which is compliant with the conditions of the process to manufacture the pellets as described herein, or of mixtures of said materials. A suitable inorganic material for the core particles is e.g. silicon dioxide (silica), in particular coarse grade silicon dioxide. Suitable organic materials for the core particles are e.g. cellulose, in particular microcrystalline cellulose ("MCC"), starch and/or carbohydrates like sucrose or lactose. Organic materials, in particular cellulose, are preferred for the core particles. Most preferred is microcrystalline cellulose. Typically, core particles of spherical or nearly spherical shape and of varying sizes are used. For pharmaceutical use, core particles of a diameter of at least 50 micrometers are usually used, e.g. of a diameter of 50 to 2000 micrometer, preferably of 150 to 1500 micrometer, for example of from 200 to 700 micrometer. Preferred lower limits of the diameter of a core particle are 50, 75, 100, 150, 200, 300, 400, 500, 700, 1000, or 1500 micrometer. Preferred upper limits of the diameter of core particle are 75, 100, 150, 200, 300, 400, 500, 700, 1000, 1500, or 2000 micrometer.

Coating layer

The pellets of the pharmaceutical compositions as described herein comprise at least one "coating layer". The coating layer or layers comprises or comprise at least one amylase but may also comprise two or more of said amylases. One amylase per coating layer is preferred. Furthermore, the coating layer(s) or other elements of the pharmaceutical compositions described herein may optionally comprise enzyme stabilizing agents and/or binding agents as described below. Still further, the coating layer(s) or other elements of the pharmaceutical compositions described herein may optionally comprise

additional conventional pharmaceutical auxiliaries and/or excipients as described below. Conventional coating materials may be used for the coating layer. The thickness of a coating layer may vary in a broad range and can e.g. be 5 to 2.000 micrometer, preferably 100 to 3.000 micrometer, more preferred 200 to 2.000 micrometer. Preferred lower limits of thickness of a coating layer are 50, 100, 200, 300, 400, 500, 700, 1000, 2000, or 3000 micrometer. Preferred upper limits of thickness of a coating layer are 100, 200, 300, 400, 500, 700, 1000, 2000 or 3000 micrometer.

However depending on the specific needs of the end product and depending on the manufacturing process other, even bigger thicknesses may be necessary/obtained. The thickness of coating layers besides others, is also dependant on the type of coating layer. For example non-functional coating layers usually are relatively thin, whereas coating layers of enzymes on a core particle usually are thicker and their thickness depends on the quantity of enzyme intended to be present per pellet, which in turn is also dependant on the diameter of the core particle. Fore example, if the core particle has a bigger diameter a thinner coating layer of enzyme is necessary to obtain a certain quantity of enzyme per pellet, as compared to the thickness of the enzyme coating layer on a smaller core particle in order to obtain the same quantity of enzyme per pellet.

The coating layers are usually applied to the core particles by common coating techniques and may be applied in several layers, e.g. in two, three, four, five or more layers, over each other, as is known in the art. One amylase coating layer is preferred.

The pellets of the pharmaceutical compositions as described herein may further comprise one or more (i.e. two, three, four, five, six, seven, eight, nine, ten, or more) additional coating layers beside the “amylase coating layer”. In case one, two or more amylase coating layers are comprised in the pellet, the pellet may optionally comprise one or more additional coating layers, e.g. for separating the amylase coating layer(s) from the surface of the core particle and/or from other amylase coating layers (“separating layer(s)”) or for providing a top coating layer applied on the surface of the amylase coating layer to protect the same from direct contact with the surrounding environment (“top coat layer(s)”). In a preferred embodiment of the invention, the top coat layer comprises

or consists of a functional (e.g. an enteric coating) coating. In another embodiment, the top coat layer comprises or consists of a so called "non-functional" coating. In case two or more coating layers are comprised in the pellet of the pharmaceutical compositions as described herein, the two or more coating layers may be applied i) in direct contact to each other or ii) may be separated from each other by the application of one or more additional coating layers (i.e. separating layers).

There are different ways to manufacture a pellet containing more than one coating layer. One option is to coat the pellets stepwise, i.e. to add a first coating layer to the pellet and then to add a second coating layer to the pellet. It might be necessary to dry the coated pellets after each coating step. In case more then two coating layers are needed the further coating layers are also added stepwise in the same or similar way.

If the pellets of the invention comprise more than one coating layer there might be various combinations of coating layers. For example there might be coating layers comprising one amylase, or coating layers which contain mixtures of more than one amylase, or which might contain mixtures of one or more amylases with one or more lipase and/or with one or more protease and/or with other substances such as enzyme stabilizing agents and/or binding agents.

Alternatively or in addition it is possible to combine different layers for example: 1. an amylase layer, 2. a separation layer, 3. a protease layer. Such an assembly of distinct layers containing different classes of enzymes (amylases, proteases, lipases, etc.) might be useful in order to physically separate these enzymes from each other, in case these enzymes interfere with each other during manufacture or during storage of the pellets. For example a protease might digest an amylase or lipase, or an amylase might remove sugar structures present in the post-translational modification of a protease or lipase, etc. Alternatively or in addition, if certain enzymes do not interfere with each other, these enzymes might be mixed and directly coated as a mixture onto a core particle resulting in for example an assembly such as: 1. a mixture of amylase and lipase, 2. a separation layer, 3. a protease layer. Alternatively coating layers containing different enzymes might be sequentially coated onto a core particle without a separation layer, if this is sufficient

to prevent the enzymes form interfering with each other. Also mixtures of different types of amylases, or mixtures of one ore more amylase with one ore more lipase, or mixtures of one or more amylase with one ore more protease, or mixtures of one or more amylase with one or more lipase and with one or more protease, or mixture of one or more lipase with one or more protease are possible. Preferably these kinds of assemblies of different coating layers are made in a way to physically separate enzymes from each other, which are not compatible to each other, whereas enzymes which are compatible to each other are combined in one coating layer. Combining two or more enzymes into one coating layer results in a less complex and less expensive manufacturing process and at the same time represents an easy way to combine the enzymes in a fixed ratio to each other.

Conventional additional coating layers and methods known in the art may be used, e.g. as described in documents DK 2002 00473, DK 2001 01930, WO 89/08694, WO 89/08695, and/or WO 00/01793. Other examples of conventional coating materials may be found in US 4,106,991, EP 170360, EP 304332, EP 304331, EP 458849, EP 458845, WO 97/39116, WO 92/12645 A, WO 89/08695, WO 89/08694, WO 87/07292, WO 91/06638, WO 92/13030, WO 93/07260, WO 93/07263, WO 96/38527, WO 96/16151, WO 97/23605, WO 01/25412, WO 02/20746, WO 02/28369, US 5,879,920, US 5,324,649, US 4,689,297, US 6,348,442, EP 206417, EP 193829, DE 4344215, DE 4322229 A, DE 263790, JP 61162185 A and/or JP 58179492, the disclosure of all of the cited documents being incorporated herein by reference.

Binding agent

Suitable "binding agents" for use with the coating layers or with other elements of the pharmaceutical compositions described herein may e.g. be agents with a high melting point or no melting point at all and optionally of a non-waxy nature. For example, cellulose derivatives may be used as suitable binding agents, in particular hydroxypropylmethylcellulose ("hypromellose"), hydroxypropylcellulose, methylcellulose or carboxymethylcellulose. Furthermore, suitable binding agents may be selected from polyvinylpyrrolidon ("PVP"), and polyvinylalcohol. Usually the binding agents are used in an amount of 0-20 % (w/w) per weight of amylase, preferably in an amount of 2.5-10% (w/w). Pref-

erably the lower amount of a binding agent is 0 %, 1%, 2 %, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, or 19%. Preferably the upper amount of a binding agent is 1%, 2 %, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19% or 20%. The use of a binding agent is optional and a binding agent may be completely omitted from the pellets. Some of the used enzyme stabilizing agents may also function as binging agent. Binding agents may also be present in coating layer(s) not containing any amylase or other enzyme, for example binding agents may be present in separation layers or may be present in functional- or in non-functional top layers.

Auxiliaries and excipients

The pellets comprising amylase optionally can contain or optionally can be used in combination with appropriate conventional pharmaceutical auxiliaries and/or excipients, preferably with conventional carriers such as lactose, mannitol, corn starch, or potato starch; with excipients such as crystalline cellulose or microcrystalline cellulose, cellulose derivatives, acacia, corn starch, or gelatins; disintegrants, such as corn starch, potato starch, or sodium carboxymethylcellulose; lubricants, such as carnauba wax, white wax, shellac, waterless colloid silica, polyethylene glycol (PEGs, also known under the term macrogol) from 1500 to 20000, in particular PEG 4000, PEG 6000, PEG 8000, povidone, talc, monolein, or magnesium stearate; and if desired, with further auxiliaries and/or excipients like diluents, adjuvants, buffering agents, moistening agents, preservatives such as methylparahydroxybenzoate (E218), colouring agents such as titanium dioxide (E171), and/or flavouring agents like saccharin, orange oil, lemon oil, and/or vanillin. Further conventional pharmaceutical auxiliaries and/or excipients according to the present invention may be selected from material such as (i) one or more carriers and/or excipients; or (ii) one or more carriers, excipients, diluents, and/or adjuvants. A particular excipient may carry out multiple functions within the composition or within the pellets. These above mentioned excipients are non-limiting examples of excipients. Preferably up to a maximum of 1%, 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% (w/w) of the total weight of the pellets or the total weight of the composition containing the pellets can be an excipient. Preferably up to a minimum of 0%, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% (w/w) of the total weight of the pellets or the total weight of the composition containing the pellets can be an excipient.

Dosage forms

Generally, depending on the medical indication in question, the pharmaceutical composition of the invention may be designed for all manners of administration known in the art, including enteral administration (through the alimentary canal) and oral administration. Oral administration forms are preferred. Thus, the pharmaceutical composition is usually in solid form, such as capsules filled with pellets, or in the form of pellets or powders. In one preferred embodiment of the invention, the oral dosage form is a capsule, which contains the pharmaceutical composition comprising pellets of the present invention. The pellets within a capsule can contain pellets of the same type, e.g. pellets only containing amylase, or pellets containing only amylase and lipase, or pellets containing only amylase and protease, or pellets containing amylase, protease and lipase. Alternatively the capsules can contain mixtures of different types of pellets, e.g. mixtures of pellets containing amylase and/or pellets containing lipase and/or pellets containing lipase, and/or pellets containing amylase and lipase, and/or pellets containing amylase and protease, and/or pellets containing amylase and lipase and protease, etc. This way it is possible to have distinct rations of amylase, lipase and protease within a capsule, thereby adjusting the ratios of these enzymes according to the needs for treatment of a distinct disease or disorder, or according to the medical needs of a distinct patient or patient group. The medical practitioner will know to select the most suitable route of administration and the most suitable composition and ration of different types of pellets, and will avoid potentially dangerous or otherwise disadvantageous administration routes and dosage forms. According to a further preferred embodiment of the present invention the inventive pharmaceutical composition may optionally be further incorporated in one or more packages selected from the group consisting of sachets, blisters or bottles. The package may contain instructions an information leaflets telling how to use the composition, for what indications the composition is intended, information about potential side effects, etc.

The total dosage of the composition depends on the medical indication and on the individual medical situation of the patient. The total dosage of amylase of the composition preferably is 8000 to 200000 units, 10000 to 150000 units, preferable 12000 to 100000 units of amylase per oral dosage form. Preferably the lower limit of amylase is

8000 units, 9000 units, 10000 units, 12000 units, 15000 units, 20000 units, 25000 units, 30000 units, 35000 units, 40000 units, 45000 units, 50000 units, 60000 units, 70000 units, 80000 units, 90000 units, 100000 units, or 120000 units of amylase per oral dosage form. Preferably the upper limit of amylase is 12000 units, 15000 units, 20000 units, 25000 units, 30000 units, 35000 units, 40000 units, 45000 units, 50000 units, 60000 units, 70000 units, 80000 units, 90000 units, 100000 units 110000 units, 120000 units, 130000 units, 140000 units, 150000 units, 160000 units, 170000 units or 180000 units of amylase per oral dosage form.

Diseases and disorders

A preferred pharmaceutical use for the pharmaceutical compositions described herein is the prevention or treatment of diseases and disorders such as digestive disorders, pancreatic exocrine insufficiency, pancreatitis, cystic fibrosis, maldigestion, diabetes type I and/or diabetes type II. Pancreatic exocrine insufficiency includes among others cystic fibrosis, chronic pancreatitis, acute pancreatitis, pancreatic cancer, pancreatectomy, gastrectomy, shwachman diamond syndrome, etc. Maldigestion includes among others starch maldigestion, bloating, flatulence, abdominal pain, diarrhea, etc. With prevention of a disease or disorder is meant to prevent the occurrence of such a disease or disorder, for example by prophylactic application of a composition or medicament which comprises amylase at a time point when said disease or disorder is not jet present or diagnosed, or when said disease or disorder is not yet present or diagnosed but is expected to occur in the future. With treatment of a disease or disorder is meant for example the application of a composition or medicament which comprises amylase at a time point when said disease or disorder is present or diagnosed or when symptoms of said disease or disorder have been noticed. Treatment may include partial or completely relieving the symptoms, partial or completely supplementing the missing or the insufficient amount of amylase in the patient, or partial or completely curing the disease.

The invention is further illustrated in the following examples which are not intended to be in any way limiting to the scope of the invention as claimed.

EXAMPLES

Example 1: Enzyme activity assays

Amylase, protease and lipase activity may be determined using the FIP assays (Fédération Internationale Pharmaceutique), 1 FIP-unit = 1 Ph.Eur.-unit (European Pharmacopoeia). These assays are described in: Fédération Internationale Pharmaceutique, Scientific Section: International Commission for the standardisation of pharmaceutical enzymes. a) "Pharmaceutical Enzymes," Editors: R. Ruyssen and A. Lauwers, E. Story Scientia, Ghent, Belgium (1978), b) European Pharmacopoeia. See also Deemester et al in Lauwers A, Scharpé S (eds): Pharmaceutical Enzymes, New York, Marcel Dekker, 1997, p. 343-385. Enzyme standards can be procured from the International Commission on Pharmaceutical Enzymes, Centre for Standards, Harelbekestraat 72, B-9000 Ghent.

Amylase FIP assay

The amylolytic activity of amylase was analyzed according to the method published in the European Pharmacopoeia 5.1 using a highly purified amylase as a reference. For determination of the amylolytic activity of the amylases for use according to the invention, the assay described by the FIP was modified. In principle, starch is hydrolysed by amylase at pH 5.8 and at constant temperature (37.0 +/- 0.1°C) in the presence of sodium chloride and calcium chloride. The reducing groups resulting from the hydrolysis react with iodine in alkaline solution and the excess of iodine is titrated with thiosulphate. One unit of amylase is defined as the amount of enzyme, which, under the defined test conditions hydrolyzes 1 micro mol of glycosidic bonds per minute.

Reagents

Sample solvent: 10 g of Sodium Chloride are dissolved in 800 mL purified water and the solution diluted to a final volume of 1000 mL.

Buffer (acetate) solution pH 5.8 (0.2 mol/L): 12 g of acetic acid, 1 g of sodium chloride and 544 mg calcium chloride are dissolved in about 800 mL of water. The pH is adjusted to pH to 5.8 using a sodium hydroxide solution (about 10 N) and the solution diluted to a final volume of 1000 mL.

Substrate solution: The substrate was soluble starch (e.g. Merck, No. 101252). The water content of each batch of soluble starch was determined upon opening of the container. To a quantity of soluble starch equivalent of 20 g of the dry substrate, 50 mL of pure (filtration/ion-exchange) water was added and mixed. This suspension was, whilst stirring continuously, added to 800 mL of boiling pure water. After cooling to room temperature the substrate solution was diluted to a final volume of 1000 mL using pure water.

Sulfuric acid (p.a.) 20 %: One volume part sulfuric acid 96 % was carefully added to 4 volume parts of water.

Acetic acid, CH_3COOH, p.a., Sodium chloride, NaCl, p.a., Calcium chloride x 2 H_2O p.a., 0.25 M sodium hydroxide, 1 M hydrochloric acid, 0.1 M iodine solution (e.g. Titrisol (Merck 9910), diluted with pure water), 0.1 M sodium thiosulphate solution.

Amylase reference:

Highly purified amylase was used as a reference standard. This reference standard was accurately weighed and dissolved in a quantity of wet ice cooled solvent resulting in a solution with titration volume of thiosulfate between 2 and 4 mL. This reference standard was used to compare activity measurements across different experiments.

Sample suspension:

An accurately weighed quantity of the test sample was dissolved in wet ice cooled solvent resulting in a solution with titration volume of thiosulfate between 2 and 4 mL.

Procedure:

Using a 300 ml Erlenmeyer flask 25 mL of substrate solution, 10 mL of buffer (acetate) solution, pH 5.8 were mixed, the flask closed with a rubber stopper and put into a water bath having a temperature of 37.0 +/- 0.1 °C. As soon as the substrate mixture had reached 37 °C 1 mL of sample suspension were added, mixed and incubated for exactly 10 minutes. The reaction was stopped by addition of 4 mL of 1 M hydrochloric acid. The Erlenmeyer flask was shaken briefly and 10 mL of 0.05 M iodine solution, 25 mL 0.25 M sodium hydroxide solution and 20 ml purified water for rinsing the inner walls

of the Erlenmeyer flask were added. The flask was stored for 15 minutes in the dark. Subsequently 4 mL of sulfuric acid (20 %) were added to the reaction. Using a microburette the solution was titrated with 0.1 M sodium thiosulfate solution until the color changes to transparency. The volume of sodium thiosulfate was noted and used for later calculation of the amylase activity present in the sample tested. The amylase reference sample was tested using the same procedure. Furthermore a blank sample was determined using the same procedure, except that the 4 mL of 1 M hydrochloric acid was added prior to the amylase-containing sample thereby inhibiting the amylase reaction. The amylase activity in Units per g of test sample [U/g] was calculated using the following equation:

$$\frac{nU - nUB \times 5 \times 1000 \text{ test solution[mL]}}{2 \text{ ml} \times \text{sample weight [mg]}} = \frac{\text{amylase [units]}}{\text{test sample [g]}}$$

nU: consumption of 0.1 M sodium thiosulphate [mL] used in the titration of the test suspension;

nUB: consumption of 0.1 M sodium thiosulphate [mL] used in the blank titration of the test suspension

Table 2: Amylase purity and activity recovery without and with presence of different amylase-stabilizing agents during spray-drying

	1	2	3	4	5
	Amylase Activity [U/g] x1000	Amylase Protein in Sample [mg/g]	Amylase Purity [HPLC Area-%]	Specific Amylase Activity [U/g	Activity Recovery [%]

				amylase] x1000	
Amylase not yet spray-dried: (liquid concentrate)	352	8.75	98,67	40229	100 by definition
Amylase, spray-dried: No stabilizer					68
Enzym-stabilizing agent:					
Sucrose (1:0.5)*	20200	509	98.74	39686	99
Sucrose (1:1)	17200	421	98.67	40855	102
Maltitol (1:0.5)	20000	509	96.63	39293	98
Maltitol (1:2)	16900	436	98.77	38761	96
Maltitol (1:3)	10300	256	98.74	40234	100
Dextran 1 (1:0.5)	20700	517	98.80	40039	100
Dextran 1 (1:1)	17300	394	99.31	43909	109
Methionin (1:0.125)	21500	581	91.87***	37005	92
Methionin (1:0.25)	21600	576	90.73***	37500	93
Methionin (1:0.5)	20800	502	85.49***	41434	103
Methionin (1:1)	14700	382	75.87***	38482	96
Hydroxyl-Propyl beta Cyclo-dextrin (1:0.1)	21600	592	98.38	36486	91
Hydroxyl-Propyl beta Cyclo-dextrin (1:0.5)	20200	521	98.63	38772	96
Hydroxyl-Propyl beta Cyclo-dextrin (1:1)	16900	423	98.62	39953	99
Glycin (1:0.5)	19200	497	93.08***	38632	96
Glycin (1:1)	16200	420	98.30***	38571	96
$CaSO_4 \times 2H_2O$(1:0.025)	22100	625	98.20	35360	88
$CaSO_4 \times 2H_2O$(1:0.05)	22800	653	98.05	34916	87
$CaSO_4 \times 2H_2O$/Methionin (1:0.025/0.5)	21500	527	85.78***	40797	101
Amylase liquid concentrate** (start material for Trehalose)	368	8.45	99.15	43550	100 by definition
Trehalose (1:1)	19500	444	98.94	43919	101

Legend for rows as numbered on top of table:

1: Amylase activity per total weight (weight of amylase + weight of stabilizing agent)

2: Amylase protein content in mg Amylase/g total weight, as determined by area-% HPLC of amylase peak in chromatogram (for details see Example 4)

3: Amylase protein content in % of total protein, as determined by area-% HPLC of amylase peak in chromatogram (for details see Example 4)
4: specific Amylase activity (1000 x row 1 / row 2)
5: specific Amylase recovery based on Amylase activity of starting material (= 100%) = 1000 x 40229 / row 4 (40229 is the 100% value for amylase activity prior to formulation)

* Expressions such as (1:0.5) depict the weight-ratio of amylase protein:amylase-stabilizing agent based on mass (w/w), e.g. Sucrose (1:0.5) means for example 1g amylase and 0.5g Sucrose
** The determination of the stability of amylase in the presence of trehalose was determined using a separate batch of amylase
*** The measred value for "amylase-purity" of those samples containing Methionin or Glycin as amylase-stabilizing agent is on average not as good as with the other amylase-stabilizing agents. This is due to the technical effect, that the amino acids Methionin and Glycin are also detected in the UV-detector and thereby the "amylase-purity" supposedly is negagtively effected. However it can be expected, that the "amylase-purity" of these samples is as good as the "amylase-purity" of the other samples.

Alternatively to the above described assay the following amylase activity assays might be used: Alternatively, the following amylase assay can be used: Substrate: Phadebas tablets (Pharmacia Diagnostics; cross-linked, insoluble, blue-coloured starch polymer, which is mixed with bovine serum albumin and a buffer substance, and manufactured into tablets), Assay Temperature: 37°C, Assay pH: 4.3 (or 7.0, if desired), Reaction time: 20 min.

After suspension in water the starch is hydrolyzed by the alpha-amylase, giving soluble blue fragments. The absorbance of the resulting blue solution, measured at 620 nm, is a function of the alpha-amylase activity. One Fungal alpha-Amylase Unit (1 FAU) is the amount of enzyme which breaks down 5.26 g starch (Merck, Amylum solubile Erg. B. 6, Batch 9947275) per hour at the standard assay conditions. A more detailed assay description, APTSMYQI-3207, is available on request from Novozymes A/S, Krogshoejvej 36, DK-2880 Bagsvaerd, Denmark.

Example 2: Determination of amylase aggregation (preparative method, HPLC)

The method is based on gel filtration chromatography in order to separate monomeric amylase and aggregates of amylase from each other. The resulting chromatogram containing peaks of monomeric and of aggregated amylase is subsequently analyzed regarding the area below each of these peaks in order to determine the relative quantities of monomeric versus aggregated amylase which are expressed in percentage. In detail a quantity of the to be analyzed amylase formulation, which amount of formulation contained about 10 mg amylase was analyzed. The amylase was dissolved in sample buffer (20mM glycin, 1mM $CaCl_2$, 200mM NaCl, pH10) resulting in a final volume of 2 ml. Subsequently a 120 ml superdex-200 gel filtration column was loaded with 2 ml sample and eluted with a flow of 1ml/min of elution buffer (20mM glycin, 1mM $CaCl_2$, 200mM NaCl, pH10) which buffer has the same composition as the sample buffer. The chromatography took place at room temperature (about 20°C). The eluate was continuously measured by an UV detector, detecting the elution of protein (measured in arbitary units), which was recorded over time. Figure 1 shows examples of such measurments. Figure 1 A to 1D depict the elution profile over a time period of 150 minutes showing the elution of monomeric amylase at around 100 min (peak area shaded in black), and the elution of aggregates between about 40 and about 90 min. Dimers and oligomers were not further distinguished (combined peak area of all aggregates shaded in grey). Figure 1 A and B show the results of this measurement of spray-dried amylase without any added enzyme stabilizing agents at day 0 after spray-drying (Fig. 1A) and after 4 weeks storage at 37°C of spray-dried amylase (Fig. 1B), as well as a spray-dried amylase/trehalose mixture (equal mass of amylase and trehalose, e.g. 1:1 [w/w] in the mixture prior to spray-drying) at day 0 after spray-drying (Fig. 1C) and after 4 weeks storage at 37°C (Fig. 1D). Addition of the amylase-stabilizing agent trehalose clearly reduced the formation of aggregates such as dimers and oligomers. Table 3 shows exemplary data of the peak-area, representing the quantity of the amylase in aggregated versus in monomerc form, as well as the increase of aggregates over the time period of 4 weeks at 37°C. For comparison a liquid amylase which was not spray-dried was determined at day 0 and after 4 weeks ad 39°C. This sample shows almost the same content of aggregates as compared to the amylase/trehalose composition, however during the 4 weeks storage at 37°C the spray-dried amylase/trealose sample seems to be more stable than the liquid amylase which was never spray-dried.

Table 3: Trehalose inhibits formation of amylase aggregates

Sample	Aggregates [%]		Increase in aggregates [%]
	Day 0	4 weeks at 37°C	
Spray-dried amylase no stabilizing agents added	21.7	61.1	182
Spray-dried amylase + trehalose (1:1)	6.7	6.9	3
Liquid amylase concentrate not spray-dried	6.2	9.9	182

Example 3: Determination of amylase aggregation (analytic method, SEC) using size exclusion chromatography (SEC)

Instrumentation

The following HPLC-equipment Autosampler G1313A, ALSTherm G1330A, Quant. Pump G1311A, UV-detector G1314A, Vacuum degasser, G1322A, HP Column Oven G1316A, 1100 control module G1323A, LAN-interface 35900E and Empower software (all from Agilent Technoloiges) or equivalent were used.

Sample preparation:

For the reference amylase a solution of 0.9 mg/mL amylase protein was prepared. Regarding the to be determined amylase-sample a suitable amount of material was weighed to obtain a signal comparable to the amylase standard preparation, taking into account the working range of the assay, which is in the range of 0.63 to 1.17 mg/mL protein present in the to be tested solution. There were always weighted two independent samples of each amylase-containing material. All amylase-containing samples were solved in 2% sodium chloride solution (1000 mL purified water, 20 g NaCl). If the to be determined amylase-sample represented pellets an appropriate quantity of pellets was weighted, added into an Erlenmeyer flask with screw cap, 10 mL solvent was added and the Erlenmeyer flask shaken for 15 to 25 min at room temperature (= 20°C). The resulting suspension was centrifuged for 10 min with at least 14000 rpm/min and the clear supernatand was used for subsequent analysis. The gel filtration standard (BIO-RAD Laboratories Inc., Catalog # 151-1901) containing Thyroglobulin (mol. weight = 670 kDa), Bovine gamma-globulin (mol. weight = 158 kDa), Chicken ovalbumin (mol. weight = 44

kDa), Equine myoglobin (mol. weight = 17 kDa) and Vitamin B_{12} (mol. weight = 1.35 kDa) was reconstituted in 0.5 mL of purified water according to the manufacturers instructions.

Column

A size exclusion column (also termed gel filtration column), length 300 mm, internal diameter 4.6 mm (or an equivalent column) filled with TSK-GEL Super SW 2000, Tosoh Bioscience, 4.0 micro meter was rinsed with 5 column volumes of purified water at 0.35 mL/min and subsequently with 5 column volumes of phosphate buffer (100 mM NaH_2SO_4 x 2 H_2O, pH adjusted to pH 6.5 using 1M NaOH filtered with 0.1 micro meter membrane). Prior to each analysis the column was tested with the gel filtration standard.

Sample mesurement

The size exclusion chromatography was done using an isocratic operation mode using poshphate buffer (100 mM NaH_2SO_4, pH 6.5) as described above. The flow rate was 0.5 mL/min at a temperature of 30°C. The injection volume of samples was 10 micro liter using an autosampler, which was maintained at a temperature of 3 to 9°C. Each gel filtration run was monitored for 20 min using an UV-detector at 280 nm wave length.

Data analysis

The retention time and peak areas of the chromatograms were determined by electronic integration using "Empower Chromatography Data Software" (Agilent Technologies/Waters). Individual peak areas were calculated in percentage by subtracting the peak area of an individual peak by the sum of all peak areas multiplied by 100. If peak area of monomeric amylase (elution at about 7 min) and of amylase aggregates (elution prior to about 7 min) are compared, the percentage of monomeric and aggregated amylase can be determined (see table 3). The amylase main compound and the sum of higher aggregated amylase and the sum of impurities were compared and the "area purity" was determined in percentage. Figure 1 shows exemplary data of amylase with and without trehalose as amylase-stabilizing agent. Figure 1 shows distinct peaks of the amylase monomers (shaded in black) and peaks representing amylase oligomers and dimeres (shaded in grey). The amylase monomer peak can also be used to determine the purity of amylase (area-% SEC) by comparing the area below the peak of the amylase monomers with the sum of the areas of all other peaks.

Table 4: Determination of amylase monomers, dimers and oligomers using size exclusion chromatography (SEC)

Enzyme-stabilizing agent (Amylase : Enzyme-stabilizing agent) (w/w)	Oligomers [%]	Dimers [%]	Monomers [%]
Only amylase; prior to spray-drying (Amylase liquid concentrate)	0.01	0.85	98.11
* Only amylase; after spray-drying → data from table 3	21.7		**78.3**
* Trehalose (1:1) → data from table 3	6.7		93.3
Sucrose (1:0.5)	0.05	1.40	98.56
Sucrose (1:1)	0.00	1.10	98.90
Maltitol (1:0.5)	0.06	1.31	98.63
Maltitol (1:2)	0.10	1.34	98.58
Maltitol (1:3)	0.11	1.39	98.51
Dextran (1:0.5)	0.05	3.10	96.86
Dextran (1:1)	0.05	3.59	96.31
Methionin (1:0.125)	2.22	1.91	95.88
Methionin (1:0.25)	2.85	1.46	95.70
Methionin (1:0.5)	1.50	1.24	97.27
Methionin (1:1)	2.48	1.55	95.98
Hydroxyl-Propyl beta Cyclodextrin (1:0.1)	3.06	1.43	95.41
Hydroxyl-Propyl beta Cyclodextrin (1:0.5)	0.52	1.42	98.06
Hydroxyl-Propyl beta Cyclodextrin (1:1)	0.00	1.13	98.87
Glycin (1:0.5)	0.06	1.03	99.02
Glycin (1:1)	0.47	1.26	98.28
$CaSO_4$ x $2H_2O$ (1:0.025)	4.59	2.86	92.56
$CaSO_4$ x 2H2O (1:0.05)	3.43	2.58	93.89
$CaSO_4$ x $2H_2O$ / Methionin (1:0.025 / 0.5)	0.35	1.13	98.53

* These data were measured using the preparative method (HPLC) for determination of amylase aggregation, as described in Example 2. The method of Example 2 does only distinguish amylase oligomers and amylase dimers but only distinguishes amylase monomers from aggregated amylase (aggregated amylase comprises amylase dimers and amylase oligomers). These data are also shown in Table 3. The value of 78.3 "Monomers [%]" was calculated based on the knowledge that the sum of amylase oligomers, dimers and monomers adds up to 100% and that the amylase oligomers and dimers in this example was determined to be 21.7 %.

Example 4: Determination of amylase content and purity

Impurities of amylase and of amylase compositions were determined using a gradient revers phase HPLC chromatography (HPLC = High Pressure Liquid Chromatogrphy). Quantitation is done using an external standard, whereas purity is determined by comparision of the area-% of the integral below the peak of the main compound (= amylase), compared with the sum of the area-% of the integral below all other (= non amylase) peaks.

Instrumentation

The following HPLC-equipment Autosampler G1313A, Quant. Pump G1311A, UV-detector G1314A, Vacuum degasser, G1322A, HP Column Oven G1316A, 1100 control module G1323A, LAN-interface 35900E and ChemServer (all from Agilent Technologes) or equivalent were used.

Sample preparation:

For the reference amylase a solution of 0.9 mg/mL amylase protein was prepared. Regarding the to be determined amylase-sample a suitable amount of material was weighed to obtain a signal comparable to the amylase standard preparation, taking into account the working range of the assay, which is in the range of 0.63 to 1.17 mg/mL protein present in the to be tested solution. There were always weighted two independent samples of each amylase-containing material. All amylase-containing samples were solved in 2% [w/w] sodium chloride solution (1000 mL purified water, 20 g NaCl). If the to be determined amylase-sample represented pellets an appropriate quantity of pellets was weighted, added into an Erlenmeyer flask with screw cap, 10 mL solvent was added and the Erlenmeyer flask shaken for 15 to 25 min at room temperature (= 20°C). The resulting suspension was centrifuged if necessary and the clear supernatant was used for analysis. For the analytical chromatography 10 micro liter of sample are injected into the reverse phase column.

Column

A reverse phase column, length 125 mm, internal diameter 3 mm (or an equivalent column) filled with YMC Protein RP, S-5 micro meter (YMC Europe GmbH, Schermbeck, germany).

Sample mesurement

The reverse phase chromatography was done using a gradient of buffer A (purified water with 0.05 % [v/v] trifluoroacetic acid (TFA)) and buffer B (acetonitrile with 0.05 % [v/v] trifluoroacetic acid (TFA), e.g. a mixture of 0.05 ml TFA with 99.95 ml acetonitrile) with a flow rate of 1 mL/min at a column temperature of 40 +/- 1 °C. The injection volume of samples was 10 micro liter using an autosampler, which was maintained at a temperature of 3 to 9°C. Each gel filtration run was monitored for 40 min using an UV-detector at 214 nm wave length. The gradient of the chromatography was as follows:

Time [min]	% buffer A	% buffer	
0	95	5	linear gradient to
35	41	59	linear gradient to
35.1	95	5	isocratic
40	95	5	Equilibration

Data analysis

A well characterized amylase reference standard was used as reference where the absolute protein content had been determined independently by amino acid analysis (assaying the content of amino acids after hydrolysis by HPLC after derivatisation). Quantification of all peaks is performed according to the area-% method and the area-% of the amylase peaks are expressed as percentage of the total area. An example of such a chromatography of an amylase sample is shown in Figure 2. The main peak is the amylase peak (Figure 1 A) whereas a few minor peaks (only clearly visible in the enlarged section of the chromatogram (Figure 1 B) represent impurities. The areas below the peaks of the chromatograms were determined by electronic integration and the sum of the areas below the peaks of the impurities and below the peak of the amylase were used to calculate the purity of the amylase in area-% (HPLC) which is a measure for the purity of the amylase. Alternatively the purity can also be determined by calculation of the areas below the peak of the amylase monomers and the sum of the areas below the peaks of amylase dimers and oligomers obtained by the method of Examples 2 and 3. This kind of purity is termed area-% (SEC).

The total weight of the analyzed amylase-containing sample was used to calculate the amylase protein content. This is a measure for the total amount of amylase protein present in the sample besides other constituents such as the amylase-stabilizing agents, lipase-stabilizing agents, protease-stabilizing agents, enzyme-stabilizing agents, core particles, salts, binding agents, substances originating from functional or non-functional coating, etc.

Example 5: Spray-drying of Amylase

Amylase liquid concentrate was poured into the feed tank of the spray dryer. The predefined amount of stabilizing agent was added to amylase liquid concentrate in the feed tank while stirring. The amylase liquid concentrate was obtained by purifying amylase from microbial amylase sources. The temperature of the feed tank was held at 5 +/- 3°C. After complete dissolution of the stabilizing agent(s), the spray-drying step was initiated. The spray-dryer was set to an inlet temperature of 140 +/- 10°C. From the feed tank, Amylase liquid concentrate was pumped into the nozzle of the spray dryer. The feeding rate was adjusted to get an outlet temperature of 75 +/- 3°C. Spray dried Amylase was collected using a cyclone and/or a filter bag.

Example 6: Praparation of coated non-pareille pellets comprising Amylase

A fluid bed coater equipped with a two-fluid nozzle and a wurster apparatus was preheated to approximately 50°C. An amount of 3 kg of core pellets (non-pareille pellets) of an average diameter of 500 micro meter made from microcrystalline cellulose were weighed and placed into the fluid bed coater and are preheated to a product temperature of approximately 50°C. The core pellets were coated by spraying the solution of amylase with added amylase-stabilizing agent on the pellets as known in the art. The spraying solution containing the amylase was kept at 5 +/- 3°C during spraying. The product temperature was controlled not to exceed 55°C, preferentially being in the range of 50 to 54°C by controlling the drying air temperature. Once the complete solution of stabilized amylase had been sprayed onto the core pellets, the heater of the fluid bed dryer was turned off and the process stopped after another five minutes. The amylase-coated non-pareille pellets were packed and stored until further use.

The solution used for the coating of the core pellets can be either prepared from amylase which has been mixed with an amylase-stabilizing agent and spray-dried, or the solution can be prepared from a liquid amylase concentrate mixed with an amylase-stabilizing agent just prior to the coating process.

As seen in Table 5 below, the further processing of a amylase of the starting material (= amylase pre-mixed with amylase-stabilizing agent and subsequently spray-dried) by coating it to core particles results in no activity loss. This indicates that the amylase-stabilizing agent present in the spray-dried amylase also protects the amylase activity during further processing of the amylase to coated pellets.

Table 5: Amylase activity present in the coating of core pellets, compared with amylase activity of the spray-dried starting material

	Amylase Purity [Area-% of SEC]	Activity recovery based on spray-dried amylase [%]
Amylase spraydried with Sucrose (1:1) = Sample 1	97.8	100*
Amylase spraydried with Sucrose (1:0.5) = Sample 2	97.3	100*
Amylase spraydried with Trehalose (1:1) = Sample 3	97.5	100*
Core particles coated with Sample 1 Sucrose (1:1)	97.2	98.5
Core particles coated with Sample 2 Sucrose (1:0.5)	97.1	102.4
Core particles coated with Sample 3 Trehalose (1:1)	97.4	101.6

* The value for "Activity recovery based on spray-dried amylase" is set to 100 %. The variation of this activity after manufacture of the coated core particles using that specific spray-dried amylase composition is measured using the method of Example 1.

Example 7: Production of pellets containing amylase manufactured by an extrusion process (not according to the invention)

Amylase and microcrystalline cellulose were dry-premixed in a mixer. After addition of isopropanol the mass was mixed and extruded with a conventional extruder through a die with holes of 0.8 mm diameter to form cylindrical pellets. The bead temperature was not exceeding 50 °C while pressing. The extrudate produced was rounded to spherical pellets with a conventional spheronizer by adding the necessary amount of isopropyl alcohol (70%). The pellets were dried at a supply temperature of approximately 40°C in a vacuum dryer (product temperature not to exceed 45°C). Separation of the dried pellets was performed using a mechanical sieving machine with 0.7 and 1.4 mm screens. The sieve fraction of ≥ 0.7 mm and ≤ 1.4 mm are collected for further processing. Over- and undersized pellets were rejected and kept for further use. These extrusion amylase pellets were compared regarding the amylase activity of the original starting material (spray-dried amylase) compared to the amylase activity of the extruded amylase pellets.

Table 6: Comparison of amylase activity of spray-dried amylase, but without enzyme-stabizing agents, before and after extrusion of pellets

	Amylase Purity [Area-% of HPLC]	Activity recovery based on spray-dried amylase [%]
Amylase: spraydried, + HPMC no amylase-stabilizing agent = Sample 1	98.1	100*
Amylase: extrusion pellets prepared from Sample 1	99.2	72.1

* By definition the starting material (spray-dried amylase) has 100 % activity

Example 8: Preparation of amylase pellets using spray-dried amylase and the rotary-agglomeration method

For this method amylase is used, which has been prepared by mixing the amylase with an amylase-stabilizing agent prior to spray-drying, thereby obtaining an amylase powder. This method so far has not been performed, but could be done as follows:

250 g of amylase in powder form and 250 g of microcrystalline cellulose (e.g. Avicel 101) are weighed and placed in a pilot scale rotary agglomerator (e.g. Glatt or VectorFreund). Setting the rotary plate to a speed of approximately 1200 rpm, the drying air temperature to 30°C and the drying air flow to 50 m^3/h, the process is started with a dry premixing

step. Using a two-fluid nozzle, water is sprayed into the moving bed at an atomization air pressure of 3 bar with a flow rate of 10-20 g/min. Samples are taken continuously and the size of the pellets is measured using e.g. sieve analysis. Once the required average pellets size of e.g. 1000 µm is reached, the water flow is stopped and the drying air temperature is raised to 50°C. The final drying step is stopped once a product temperature of 40°C is reached. The product is sieved to remove fine and coarse particles and then is packed and stored until further use

Example 9: Preparation of amylase pellets using liquid amylase and a rotary-agglomeration apparatus

For this method amylase liquid concentrate is used, which has been mixed with an amylase-stabilizing agent, but which has not been spray-dried prior its use. This method so far has not been performed, but could be done as follows:

The amylase liquid concentrate is poured into a cooled feed tank of a rotary agglomerator. The temperature of the feed tank is held at 5 +/- 3°C. The predefined amount of stabilizing agents is added to Amylase liquid concentrate in the feed tank while stirring. The temperature of the feed tank is held at 5 +/- 3°C. After complete dissolution of the stabilizing agent(s), the pelletizing step can be initiated. In parallel, 500 g of microcrystalline cellulose (e.g. Avicel 101) are weighed and placed in a pilot scale rotary agglomerator (e.g. Glatt or VectorFreund). Setting the rotary plate to a speed of approx. 1200 rpm, the drying air temperature to 30°C and the drying air flow to 50 m³/h, the process is started. Using a two-fluid nozzle, the solution of purified amylase containing the stabilizing agent(s) is sprayed into the moving bed at an atomization air pressure of 3 bar with a flow rate of 10-20 g/min. Samples are taken continuously and the size of the pellets is measured using e.g. sieve analysis. Once the required average pellets size of e.g. 1000 µm is reached, the liquid flow is stopped and the drying air temperature is raised to 50°C. The final drying step is stopped once a product temperature of 40°C is reached. The product is sieved to remove fine and coarse particles and then is packed and stored until further use.

Example 10: Determination of reducing sugars

Reducing sugars may be detected by use of the Maillard reaction and subsequent detection of the reaction products. The carbonyl group of the sugar reacts with the amino

group of the amino acid, producing N-substituted glycosylamine and water. At higher temperatures and lower moisture (water) conditons the Maillard reaction is promoted. The resulting reaction products depend on the type of sugar and amino acid reacting with each other and are determined with common analytic methods known in the art such as reverse-phase HPLC (High Pressure Liquid Cromatography) and mass spectromety, for example ESI-MS (Electro Spray Ionisation-Mass Spectrometry). Figure 3 shows the reaction scheme of an exemplary Maillard reaction.

Claims

1. A pharmaceutical composition comprising a spray-dried mixture of amylase and at least one amylase-stabilizing agent, said spray-dried mixture being obtainable by spray-drying a liquid comprising an amylase and at least one amylase-stabilizing agent, wherein said amylase shows at least 85% of the specific amylase activity relative to the specific amylase activity before spray-drying.

2. The pharmaceutical composition according to claim 1 in the form of a powder, in the form of a granulate, in the form of pellets or in the form of a coating on a core particle.

3. The pharmaceutical composition according to claim 2 further processed into a tablet, a capsule or a sachet.

4. The pharmaceutical composition according to any one of claims 1 to 3 wherein said amylase-stabilizing agent is a non-reducing carbohydrate, preferably a polysaccharide, an oligosaccharide, a disaccharide, a sugar-alcohol, or a cyclodextrin.

5. The pharmaceutical composition according to any one of claims 1 to 4, wherein
   (a) said enzyme stabilizing agent is a polysaccharide selected from Inulin, or Dextran, preferably Dextran 1, Dextran 40 or Dextran 70,
   (b) said enzyme stabilizing agent is an oligosaccharide selected from Sucrose or Trehalose,
   (c) said enzyme stabilizing agent is a sugar-alcohol selected from Sorbitol, Mannitol, or Maltitol,
   (d) said enzyme stabilizing agent is the cyclodextrin Hydroxyl-Propyl beta Cyclodextrin, or
   (e) said enzyme stabilizing agent is selected from Gycine, Arginine or a mixture of Calcium salt and Methionine.

6. The pharmaceutical composition according to any one of claims 1 to 5 wherein said amylase is selected from alpha-amylase, beta-amylase, alpha-glucosidase, isoamylase, glucan 1,4-alpha-maltohydrolase, preferably an amylase originating

from bacteria, yeast, mammalian or human cells, preferably an amylase produced by recombinant technologies.

7. The pharmaceutical composition according to any one of claims 1 to 6, wherein said amylase is purified and has a purity of at least 90 area-% (w/w).

8. The pharmaceutical composition according to any one of claims 1 to 7, furthermore comprising a lipase and/or a protease.

9. Pellets, granulates, coated core-particles or tablets according to claim 2 or 3, coated with at least one layer of a pharmaceutically acceptable substance.

10. Pellets, granulates, coated core-particles or tablets according to claim 9, wherein said at least one layer is
    (a) a functional coating, preferably an enteric coating protecting the enzymes of the pellets from acid pH during gastric passage, and/or
    (b) a non-functional coating, preferably a coating preventing the formation of dust from the material positioned below said non-functional coating.

11. Method for the manufacture of a pharmaceutical composition according to claim 1 comprising the step of:
    (a) spray-drying an amylase solution mixed with at least one amylase-stabilizing agent prior to spray-drying
    resulting in a pharmaceutical composition comprising an amylase showing at least 85% of the specific amylase activity relative to the specific amylase activity prior to spray-drying.

12. Method for the manufacture of a pharmaceutical composition comprising the step of:
    (a) coating pharmaceutically acceptable core particles with a coating solution comprising amylase, wherein said coating solution is prepared by mixing of an amylase solution with at least one enzyme stabilizing agent and optionally coating said core particles with one or more further coating layer comprising

amylase and/or other pharmaceutically acceptable substances and incorporating said coated core pellets into a pharmaceutical composition.

13. The method according to claim 13, wherein said coating solution is prepared from a spray-dried amylase according to claim 1 and at least one solvent, wherein said amylase has been mixed with said at least one amylase-stabilizing agent prior to drying of said amylase.

14. Method for the manufacture of a pharmaceutical composition comprising the step of:
    (a) forming said spray-dried amylase according to claims 1 into a granulate, pellet, coated core particle, tablet, capsule or sachet

15. Pharmaceutical composition according to any one of claims 1 to 7 for the prevention or treatment of digestive disorders, pancreatic exocrine insufficiency, pancreatitis, cystic fibrosis, maldigestion, diabetes type I or diabetes type II.

Fig. 1 A-D

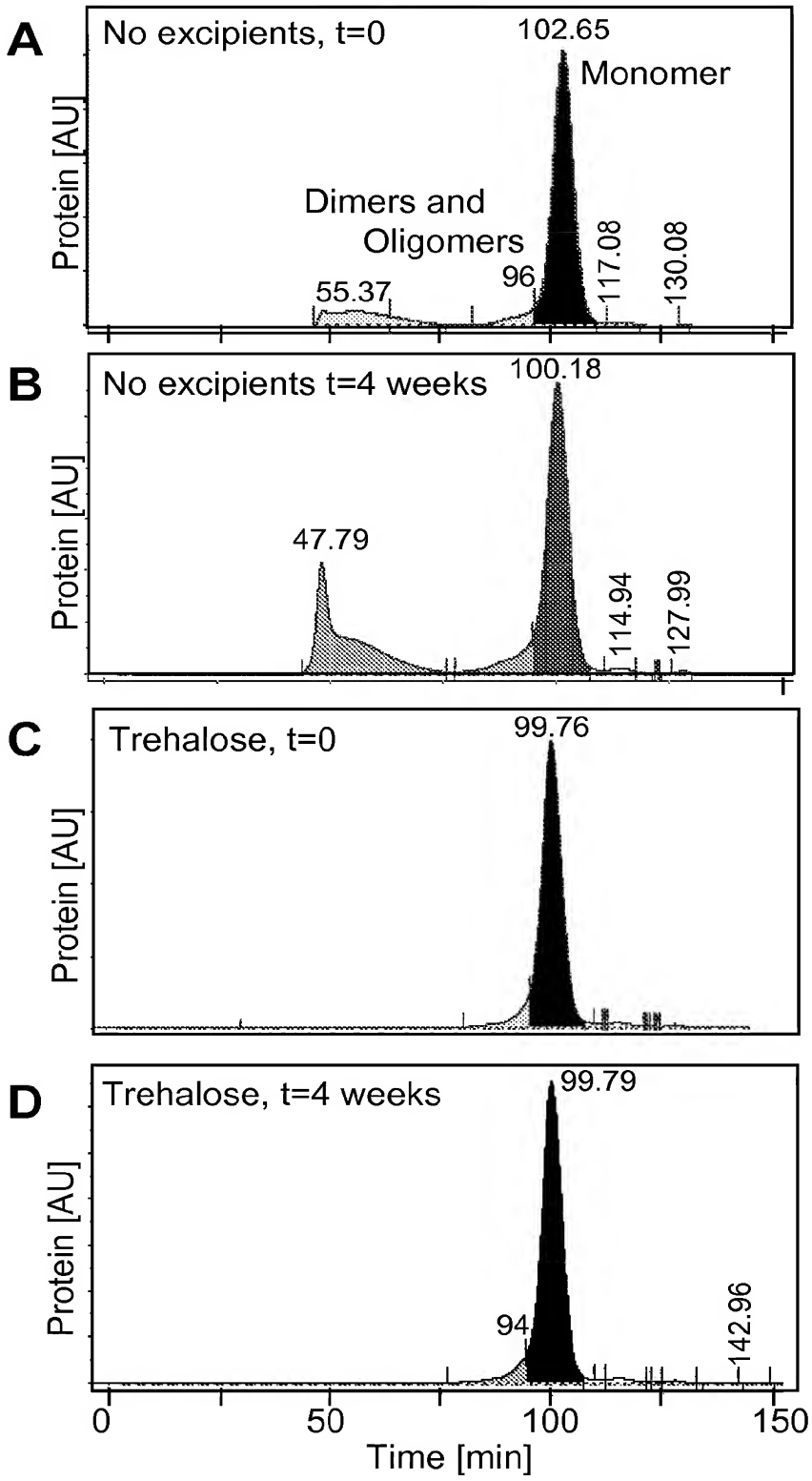
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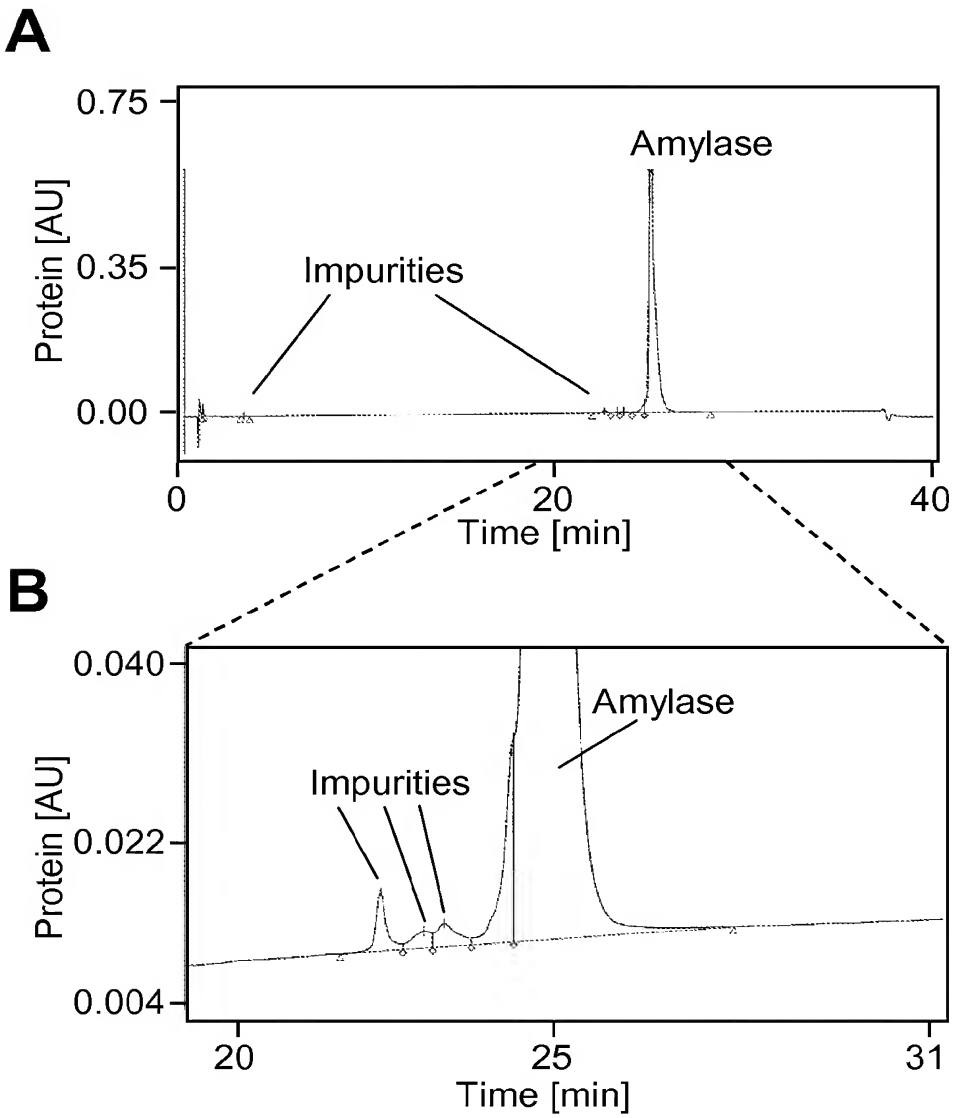
Fig. 2 A-B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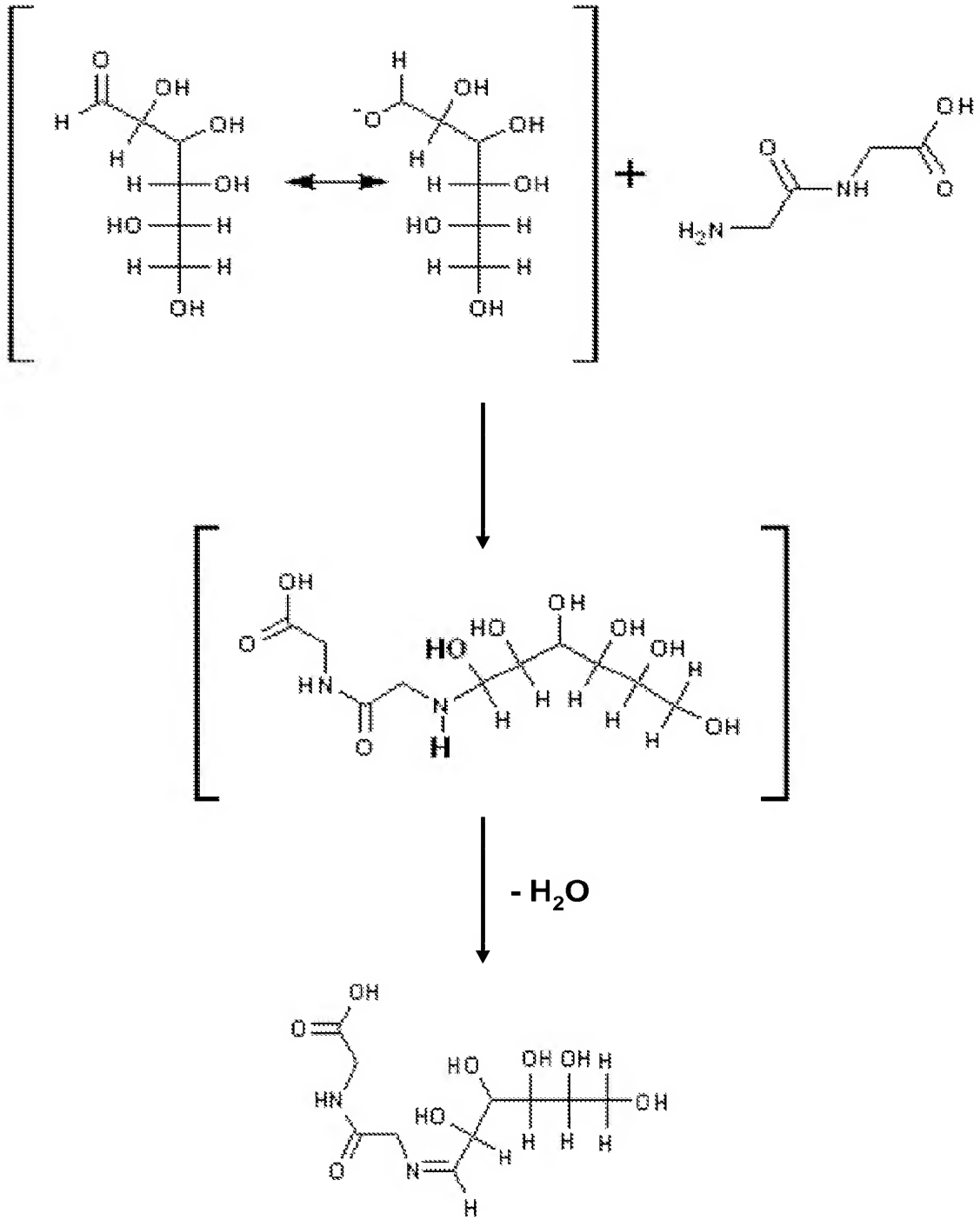
Time [min]

Fig. 3



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/059389

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N9/26 A61K9/16 A61K38/47
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 42 03 315 A1 (KALI CHEMIE PHARMA GMBH [DE]) 20 August 1992 (1992-08-20) claim 12	1,2,4-6, 8,15
Y	WO 2006/136161 A2 (NOVOZYMES AS [DK]; SOLVAY PHARM GMBH [DE]; SVENDSEN ALLAN [DK]; GREGOR) 28 December 2006 (2006-12-28) cited in the application page 19, line 13 - line 18 example 5	1-15
Y	WO 2008/102264 A2 (EURAND PHARMACEUTICALS LTD [IE]; ORTENZI GIOVANNI [IT]; MARCONI MARCO) 28 August 2008 (2008-08-28) cited in the application claims 1-132	1-15

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 August 2010	18/08/2010

Name and mailing address of the ISA/	Authorized officer
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International application No
PCT/EP2010/059389

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